J. Pharm. Pharmacol., 1965, 17, 329–340 Received January 28, 1965

Research Papers

Infrared identification of pharmaceutically important steroids with particular reference to the occurrence of polymorphism

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The infrared absorption spectra of steroids, when compared with the spectra of Authentic Specimens, provide a simple and complete means of identification, provided that the effects of polymorphism are precluded. Of 35 substances examined, 16 showed no evidence of polymorphism and a further twelve were sufficiently soluble to be examined in solution. Specific solvent treatments, details of which are given, may be necessary with the remaining seven substances if the spectra of the sample and of the Authentic Specimen are not identical when first examined.

THE establishment of a collection of Authentic Specimens for use with certain tests of the British Pharmacopoeia and the British Pharmaceutical Codex prompted an investigation into the incidence of polymorphism in a number of pharmaceutically important steroids. Thirty-four of the substances examined were required for the preparation of infrared spectra to be used for comparison purposes in qualitative identification tests. The 35th substance, digitoxin, has not been issued as an Authentic Specimen but was included for comparison with digoxin. It was therefore necessary to establish conditions whereby different forms of a substance, should they exist, might be converted to a single form thus eliminating differences in solid-state infrared spectra.

Polymorphism in steroids has been known for at least 30 years and differences in infrared spectra of different forms have been reported (e.g. Dickson, Page & Rogers, 1955; Smakula, Gori & Wotiz, 1957; Callow & Kennard, 1961). Changes in spectrum due to grinding with potassium bromide have also been reported to occur with steroids (Roberts 1957; Hayden & Sammul, 1960); such changes have also been reported with other compounds containing hydroxyl groups (Barker, Bourne, Neely & Whiffen, 1954; Farmer, 1957). In some instances changes in spectrum were ascribed to conversion of a crystalline form into an amorphous form or into a second crystalline form (Baker, 1957). Changes in crystalline form and thus in the absorption spectrum might also be induced by the solvent extraction methods used for the isolation of steroids from pharmaceutical preparations before infra-red examination.

Experimental

MATERIALS

Chloroform, acetone and ethanol used for solvent treatments were of B.P. quality. Samples were prepared for infrared examination using

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liquid paraffin B.P., potassium chloride A.R. or potassium bromide (spectroscopic grade: E. Merck A. G., Darmstadt).

Except where otherwise stated, the steroid samples examined were Authentic Specimens provided by the British Pharmacopoeia and the British Pharmaceutical Codex, and the names used are those under which these specimens are issued.

The chloroform contains up to 2% of ethanol, but no evidence was found to suggest that this affected the crystalline state of substances recovered from chloroform.

SOLVENT TREATMENTS

Each sample was subjected to the following solvent treatments: separate portions were dissolved in minimum amounts of chloroform, of acetone and of ethanol and the solutions evaporated to dryness on a water-bath. Further portions were dissolved in chloroform and also in acetone in an agate mortar and the solvent allowed to evaporate in a current of air at room temperature. Where necessary the last traces of solvent were removed with a jet of air. These solvent treatments will be referred to hereafter as hot chloroform, hot acetone, hot ethanol, cold chloroform and cold acetone. In certain instances, noted below, additional solvents were used. Digoxin, hydrocortisone sodium succinate and prednisolone sodium phosphate were insoluble in chloroform and acetone, and could only be recrystallised from ethanol (50% aqueous ethanol in the last case).

INFRARED ABSORPTION SPECTRA*

Spectra of all the steroids, before and after solvent treatment, were obtained in the solid phase using either a mull in liquid paraffin or an alkali halide disc. Where possible, spectra were also recorded from solutions in chloroform. The procedures used were as recommended in Appendix IV. I. of the British Pharmacopoeia, 1963. Materials for examination as mulls were ground lightly by hand in a mortar before adding liquid paraffin; those used for disc preparation were ground with potassium bromide or potassium chloride for 2 min in a ball mill to ensure thorough mixing before pressing.

All the spectra were obtained by one of us (R.J.M.) using a Grubb Parsons GS 2 grating spectrometer. A Unicam SP 200 spectrometer with sodium chloride prism was used (by C.A.J.) to confirm many of these independently, and also to examine additional samples of many of the substances.

The presence of discrete polymorphic forms was confirmed by means of powder diffraction patterns recorded on a Metropolitan-Vickers Raymax 100 instrument using a 9 cm camera and cobalt K α-radiation.

Results and discussion

Table 1 lists the substances examined and shows those found to exist in more than one solid form and those sufficiently soluble in chloroform

*Throughout the paper the use of the word "spectrum" refers to the infrared absorption spectrum.

TABLE 1. INCIDENCE OF POLYMORPHISM IN THE 35 SUBSTANCES EXAMINED

Substance	No. of solid forms identified	Solution Spectrum possible
Betamethasone†	2	No.
Cortisone acetate†	7	Yes
Deoxycortone acetate	1	No
Deoxycortone trimethylacetate	1	No
Dexamethasone	1	No
Dexamethasone acetate†	4	Yes
Digitoxin†	1	No
Digoxin	1*	No
Dimethisterone	2	Yes
Ethinyloestradiol†	3	Yes
Ethisterone	1	No
Fludrocortisone acetate†	4	No
Fluocinolone acetonide†	2	Yes
Fluoxymesterone	1	No
Hydrocortisone	2	No
Hydrocortisone acetate	1	No
Hydrocortisone hydrogen succinate	1	No
Hydrocortisone sodium succinate	1	No
Methylprednisolone	2	No
Methyltestosterone	1	Yes
Norethandrolone	2	Yes
Norethisterone	1	No
Norethynodrel	2	Yes
Prednisolone	2	No
Prednisolone acetate	1	No
Prednisolone sodium phosphate	1	No
Prednisolone trimethylacetate	3	Yes
Prednisone	2	No
Prednisone acetate	2	Yes
Progesterone	2	Yes
Sprironolactone†	3	Yes
Testosterone	3	Yes
Testosterone propionate	1	Yes
Triamcinolone†	2	No
Triamcinolone acetonide†	1	No

* A possible second form differed only in one band.
† Differences observed between halide disc and paraffin mull spectra.

for spectra to be obtained in solution. The path length is limited by solvent absorption and in our opinion should not exceed a nominal 0·2 mm; the minimum solubility requirement was therefore about 3%. Table 1 also indicates those compounds in which differences were observed between spectra from a potassium halide disc and those from a liquid paraffin mull of the sample as received; for the most part, the other forms were examined only as mulls.

The publication of reference spectra of steroids is of limited value where several different spectra are possible. Where only one form has been detected however, its spectrum may be regarded as a reliable means of identification, though the possibility of other forms occurring must not be overlooked. Spectra for seven of the steroids examined, and for which we have detected only one solid form, have not previously appeared in readily available literature, and these are reproduced in Figs 1 and 2. The substances exhibiting polymorphism are considered individually below.

Betamethasone. Material recovered from chloroform solution gave the same spectrum as the original form; evaporation of ethanol, acetone and methanol solutions yielded increasing proportions of a second form, but this was not obtained in a pure state. The X-ray powder diffraction pattern of the mixture obtained from methanol solutions showed only lines due to the original material so the second form is presumably

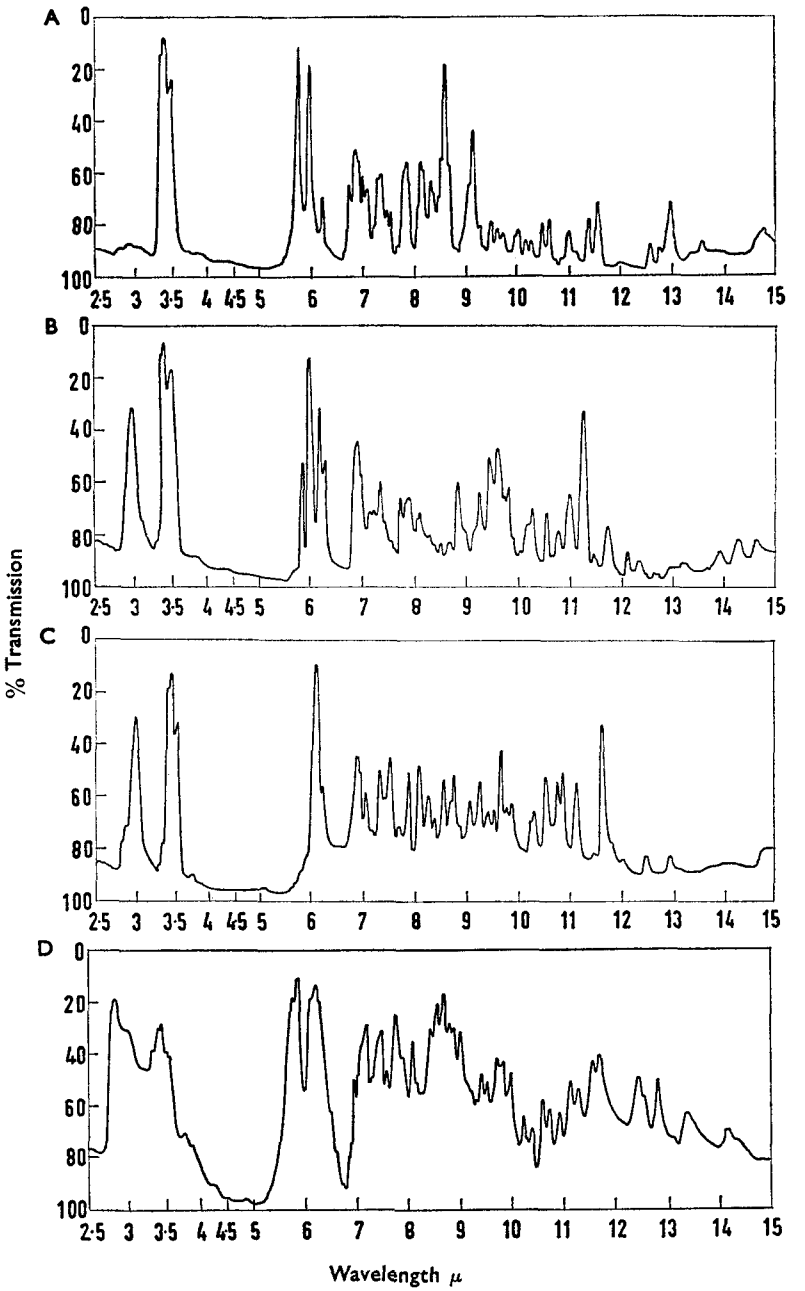

FIG. 1. A. Infrared spectrum of deoxycortone trimethylacetate (liquid paraffin mull). B. Infrared spectrum of dexamethasone (liquid paraffin mull). C. Infrared spectrum of fluoxymesterone (liquid paraffin mull). D. Infrared spectrum of hydrocortisone hydrogen succinate (potassium bromide disc).

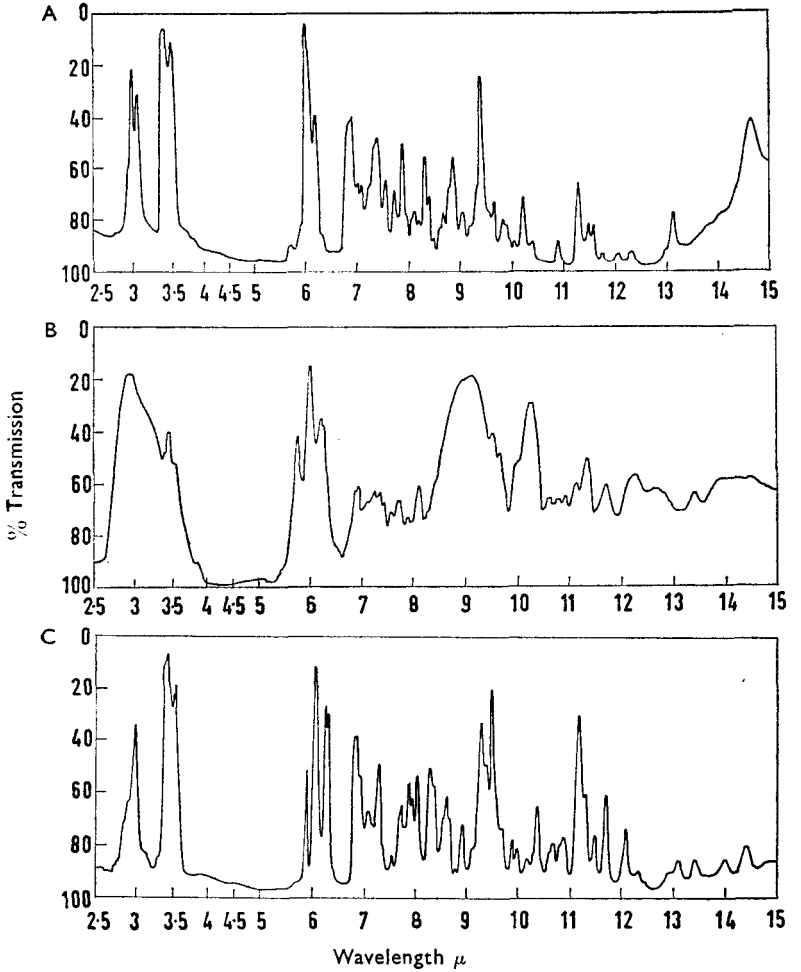

FIG. 2. A. Infrared spectrum of norethisterone (liquid paraffin mull). B. Infrared spectrum of prednisolone sodium phosphate (potassium bromide disc). C. Infrared spectrum of triamcinolone acetonide (liquid paraffin mull).

amorphous. A potassium bromide disc prepared from the original material also showed the presence of this second form.

Cortisone acetate. Callow & Kennard (1961) published X-ray diffraction patterns and partial infrared spectra of five forms. The complete spectrum of form II was published as this form was said to be reproducibly obtained by evaporation of a chloroform solution to dryness. In the present work, evaporation of chloroform solution in a beaker on a water bath gave form III, evaporation in a mortar at room temperature gave form II, and evaporation in a beaker at room temperature gave a mixture of both these forms. Ethanol, hot or cold, gave mixtures of forms I and IV, methanol gave mixtures of forms II and V, and acetone under various

conditions produced forms I, II, III, IV and a new form not described by Callow & Kennard. A mixture of the new form with form I, obtained by evaporation of acetone solution at room temperature, gave an X-ray powder diffraction pattern similar to that recorded by Beher, Parsons & Baker (1955). Many of these mixtures could not be identified solely from their infrared spectra, and X-ray patterns were used to provide confirmation. A seventh form was recovered from tetrahydrofuran solution. Of the specimens examined, the B.P. Authentic Specimen was received as form II; the U.S.P. Reference Standard and the W.H.O. Authentic Chemical Substance were both form III.

Published spectra from other sources show considerable variation: Neudert & Röpke (1957) give form II, agreeing with the spectrum published by Callow & Kennard, while Tarpley Yudis, Manowitz, Horrigan & Weiss (1954) give form I; Hayden, Sammul, Selzer & Carol (1962) who say their material was obtained by evaporation of ethanol solution, give form I but with a peak at 868 cm^{-1} apparently due to form IV; a similar spectrum is included in the Sadtler Pharmaceutical collection; and Meda (1958) shows a spectrum between 1600 and 1800 cm^{-1} which is probably a mixture of forms II and III, but might be a mixture of forms I and IV.

It is clear from this confusion that when any doubt exists about the authenticity of a sample of cortisone acetate it is advisable to record the spectrum in solution.

Dexamethasone acetate. Four forms have been distinguished one of which apparently contains chloroform. None of the solvent treatments used consistently gave a single form, though the material obtained from hot acetone closely resembled the original sample. Solubility in chloroform is only about 3% but this is sufficient to give a characteristic spectrum in a 0·2 mm cell.

Digitoxin. Hayden & others (1962) reported that a potassium bromide disc containing digitoxin showed marked changes in spectrum on heating. This statement has been confirmed, but the heated disc was much more opaque and attenuation of the reference beam was necessary to obtain a good spectrum. A liquid paraffin mull gave a spectrum similar to that of the heated disc and this technique seems preferable; Bell (1960) obtained a comparable spectrum from a potassium bromide disc, the materials being ground together by hand; there was no mention of subsequent heating. The Sadtler Pharmaceutical collection includes a spectrum of a potassium bromide disc in which the material is apparently amorphous and which is almost indistinguishable from the corresponding spectrum of digoxin. A spectrum of a liquid paraffin mull has been published in the Sadtler Standard collection. No evidence of polymorphism was found on recrystallisation.

Digoxin. The spectra of the Authentic Specimen in potassium bromide and in liquid paraffin mull, agree with those published by Bell (1960) and by Hayden & others (1962), though the latter authors stated that the characteristic spectrum was produced only when the disc was heated. The Sadtler Pharmaceutical collection has a spectrum of apparently

amorphous material not readily distinguishable from digitoxin (see above). Digoxin was virtually insoluble in chloroform and acetone, but recrystallisation from hot ethanol gave material with a spectrum identical to that of the original sample except for an additional small peak at 1656 cm^{-1}. This disappeared on heating and was presumably due to hydration.

Dimethisterone. Only one crystalline form was identified, the original material being recovered unchanged from cold acetone. All other solvent treatments gave a glassy solid with a different spectrum. X-ray diffraction confirmed that this was amorphous.

Ethinyloestradiol. Spectra of two crystalline forms have been recorded as mulls by Röpke & Neudert (1959), who also state that, when the potassium bromide disc technique is used, both forms give the same spectrum, which is similar to the solution spectrum. However, the same authors (Neudert & Röpke, 1957) have published a spectrum obtained from a potassium bromide disc which corresponds to their form A. On the other hand, Hayden & others (1962), using a potassium bromide disc, obtained a spectrum intermediate between the two forms, and an identical spectrum has been published by Carol (1957).

This apparent inconsistency in results is explained by the present work in which it was found that form A, the form in which the B.P. Authentic Specimen was received, is stable when ground with potassium bromide, whereas form B, obtained from hot ethanol or cold acetone evaporation, is unstable. A sample of the U.S.P. Reference Standard material was shown by both infrared spectrum and X-ray diffraction pattern to be a mixture of both forms, with B predominating. A third, amorphous form was obtained from hot acetone, and from the similarity in spectrum it seems probable that it is this which is present in pressed discs prepared from form B. Treatment with cold chloroform, recommended in the original leaflet (ASL. 18) issued with the B.P. Authentic Specimen, sometimes gave an amorphous product containing residual chloroform, and the use of solution spectra, as advised in the current leaflet (ASL. 18/2), therefore seems preferable.

Fludrocortisone acetate. Three crystalline forms and one amorphous form have been identified. No simple treatment consistently gave the same form, and interconversion between some of the forms appeared to take place spontaneously. It was found that a reasonably reproducible spectrum could be obtained by evaporating a chloroform solution in a beaker at room temperature to give a glassy material containing chloroform; on heating in an oven at 100° for 15 min the residual solvent was removed, leaving a crystalline product. The Sadtler Pharmaceutical collection includes spectra of two samples: one corresponds to the Authentic Specimen and the other to the amorphous form.

Fluocinolone acetonide. Two crystalline forms were obtained. Treatment with hot ethanol converted the original material into the second form, whilst all other solvent treatments gave mixtures of the two forms. A spectrum was also recorded from a solution in chloroform.

Hydrocortisone. The original form was recovered unchanged from hot ethanol and hot and cold acetone. On evaporation of chloroform solution the spectrum was unchanged except for additional bands at 761 and 752 cm^{-1}, apparently due to residual chloroform. X-ray diffraction measurements however, showed that it was a different crystalline form; on heating it lost chloroform and reverted to the original form. Spectra published by Antonucci, Bernstein, Heller, Lenhard, Littell & Williams (1953), Roberts, Gallagher & Jones (1958), Hayden & others (1962), and the Sadtler Pharmaceutical collection, and band positions quoted by Heller (1959) all correspond to the original form. However, a spectrum published by Hayden (1955), described as 17-hydroxycorticosterone, differs from the forms obtained in the present work.

Methylprednisolone. Two forms have been described by Higuchi, Lau, Higuchi & Shell (1963). The Authentic Specimen corresponded to their form I, which was also recovered from cold acetone and chloroform. Hot acetone yielded form II, and other solvent treatments gave mixtures. The spectrum in the Sadtler Pharmaceutical collection is of form I.

Norethandrolone. The original material was recovered unchanged from hot chloroform. Hot ethanol treatment gave a second form and all other treatments gave mixtures. A spectrum of the second form is included in the Sadtler Pharmaceutical collection.

Norethisterone. We have found no evidence of polymorphism, but the only previously published spectrum, that in the Sadtler Pharmaceutical collection, differs from that of the Authentic Specimen.

Norethynodrel. This substance, which has a double bond in the 5, 10-position, readily undergoes isomerisation to norethisterone where the double bond is between C_4 and C_5. The possibility that such isomerisation might be caused by grinding with halide or liquid paraffin has been examined but no evidence that it occurs to any significant extent has been obtained. Similarly, solution in chloroform B.P. or in methylene chloride does not induce isomerisation, but there is evidence that it begins to occur in chloroform containing as little as 0·005% w/v of hydrochloric acid. In more acid solution isomerisation may take place rapidly and to a marked extent. It is for this reason that the leaflet accompanying the B.P.C. Authentic Specimen directs that solution spectra be recorded using a solution in chloroform containing 0·1% of pyridine.

In the investigation of solid state spectra it was found that the original material was recovered unchanged from hot ethanol, hot chloroform and hot methylene chloride (the latter solvent is that recommended for recrystallisation in the leaflet issued with the B.P.C. Authentic Specimen). Evaporation of acetone, either hot or cold, yielded a second form which differs from the original only in having a strong absorption at 1645 cm^{-1}. This suggests that it may be a hydrate.

Prednisolone. The B.P. Authentic Specimen was received in one form (designated A); a sample of the U.S.P. Reference Standard material was received in a second form (B). Another commercial sample was shown by infrared spectrum and X-ray diffraction pattern to be a mixture of the

two forms. Form B was converted to form A by evaporation of hot acetone or hot chloroform solutions. When form A was dissolved in methanol and heated on a water-bath the resulting material was a mixture; a second treatment with methanol further increased the proportion of form B, though form A was still present. Heating the methanol solution under reflux for 20 min before evaporation still gave a mixture. On the other hand, when form B was dissolved in methanol and the solvent evaporated the product was entirely form B. Two recrystallisations from ethanol converted form A completely into form B.

The spectrum published by Roberts, Gallagher & Jones (1958) is of form A. Hayden & others (1962), using material recrystallised from ethanol, give a spectrum of a mixture in which form A predominates. The Sadtler Pharmaceutical collection contains two spectra, one similar to that of Hayden & others, the other predominantly of form B.

Prednisolone trimethylacetate. Three forms were identified. The original form (A) was recovered from hot acetone treatment of forms A and B (conversion from C was not investigated). Forms B and C were obtained from hot ethanol and hot chloroform respectively, but in each case required two recrystallisations. A solution spectrum was also obtained using a saturated (5%) solution in chloroform in a 0·2 mm cell.

Prednisone. The original form was recovered unchanged from all solvent treatments except cold chloroform. This gave a second crystalline form with a spectrum which differed from the original in several respects, particularly in the presence of a strong band at 749 cm^{-1}, suggesting the presence of chloroform in the crystal. Spectra published by Roberts & others (1958), Hayden & others (1962) and in the Sadtler Pharmaceutical collection all correspond to the first form.

Prednisone acetate. The original form was recovered from hot ethanol. Hot acetone and hot and cold chloroform all yielded a second form, while cold acetone gave a mixture. A solution spectrum was also obtained.

Progesterone. The original form (designated A), which has a strong absorption at 870 cm^{-1}, was recovered from cold chloroform. Hot ethanol and cold acetone gave form B, in which the strong band is at 864 cm^{-1}, while other solvent treatments gave mixtures. Spectra published by Neudert & Röpke (1957), Meda (1958) and the Sadtler Standard and Sadtler Pharmaceutical collections are all of form A; Morcillo & Alduma (1957) give form B; and spectra of mixtures in which form B predominates are given by Hayden & others (1962), who obtained their material by evaporating an ethanol solution, and by Furchgott, Rosenkrantz & Shorr (1947). The latter authors, working before the advent of the pressed disc technique, used a film deposited on a rock salt plate from pyridine solution. Progesterone is readily soluble in chloroform, and spectra can also be obtained from solutions in carbon tetrachloride and carbon disulphide.

Spironolactone. The original form was not recovered from any of the five solvent treatments. A second crystalline form was obtained from cold acetone, but all other solvent treatments gave an amorphous, glassy

material with a different spectrum. A potassium bromide disc of the original material gave a spectrum corresponding to the amorphous form. A spectrum was also obtained in chloroform solution. The Sadtler Pharmaceutical collection contains a spectrum which corresponds to a mixture of the two crystalline forms.

Testosterone. Three forms were encountered, two of which show spectral differences corresponding to those between the two forms of progesterone. The original form (A) has an absorption band at 870 cm^{-1}, and was recovered unchanged from hot acetone and hot chloroform. Cold acetone gave form B, in which the strong band is at 864 cm^{-1}, whilst form C, in which it has shifted to 881 cm^{-1}, was obtained together with form A from cold chloroform. Hot ethanol gave a mixture of forms A and B.

Published spectra show some confusion: the spectrum in the Sadtler Pharmaceutical collection shows a mixture of forms A and C, the Sadtler Standard collection includes two spectra, a rather poor mull of form A (No. 727) and a potassium bromide disc which shows a mixture of form B with a little of form A (No. 13203); a similar mixture is given by Furchgott, Rosenkrantz & Shorr (1946) using a film deposited from pyridine; the spectrum of Morcillo & Alduma (1957) is apparently of form B; that of Neudert & Röpke (1957) is of form A. Rosenkrantz, Potvin & Skogstrom (1958) use a band at 872 cm^{-1} for the quantitative estimation of testosterone in potassium bromide discs. This presumably refers to form A, but errors could obviously arise if other forms were present.

As with progesterone, spectra may be recorded in solution in chloroform, carbon tetrachloride and carbon disulphide.

Triamcinolone. The original form (A) was not recovered unchanged, though cold acetone treatment produced a spectrum fairly close to the original. Hot ethanol and hot acetone both gave a second form (B). Evaporation of a methanol solution is recommended in the leaflet issued with the B.P.C. Authentic Specimen, but this seems a bad choice as it gave mixtures of varying proportions of Forms A and B, starting from either A or B. A spectrum published in the Sadtler Pharmaceutical collection is mainly form A, but contains some impurity.

General discussion

The results quoted above show that sample preparation techniques can have a profound effect on the spectra of steroids. The inconsistency of published spectra confirms this, and serves to emphasise the necessity for comparison between the sample and an Authentic Specimen under identical conditions as the basis of any identification by infrared spectroscopy.

Experienced workers in this field (Jones & Dobriner, 1949; Page, 1957) have recommended that whenever possible infrared spectra of steroids should be recorded in solution, and with compounds which exhibit polymorphism this is undoubtedly advisable. Nevertheless, if two samples are found to give identical spectra in the solid phase, this constitutes reasonable proof of identity and no recourse to solutions is required.

Difficulty arises only when samples, which are thought to be of the same substance, give different spectra in the solid phase. Spectra recorded in solution will soon resolve any doubts, but unfortunately many of the physiologically important steroids are only sparingly soluble in the commonly used solvents, and the use of solid phase spectra is often unavoidable.

The normal way to overcome the effects of polymorphism is to convert both samples into the same form by recrystallisation from the same solvent. However, where the presence of impurities may be in question it is necessary to recover the whole of the material and solutions must therefore be evaporated to dryness. With some solvents this may lead to the production of two different crystalline forms from the same solution, and such solvents should be avoided. In many instances the temperature at which the solvent is removed can also affect the form of the resulting solid. To ensure reproducibility it has been found necessary to prescribe individual solvent treatments for the insoluble substances which exhibit polymorphism, as follows: Betamethasone: Dissolve in chloroform and evaporate on a water-bath. Fludrocortisone acetate: Dissolve in chloroform in a beaker and evaporate in a current of air at room temperature; heat residue in oven at 100° for 15 min. Hydrocortisone: Dissolve in acetone and evaporate on a water-bath. Prednisolone: Dissolve in acetone or chloroform and evaporate on a water-bath. Prednisone: Dissolve in acetone and evaporate on a water-bath. Triamcinolone: Dissolve in acetone and evaporate on a water-bath.

With regard to sample form, mulls in liquid paraffin gave more consistent spectra than potassium halide discs, particularly with the substances marked † in Table 1, and this technique appears preferable for comparison purposes (bearing in mind the presence of absorptions due to the liquid paraffin). On the other hand, prednisolone sodium phosphate was difficult to grind in liquid paraffin and gave a much better spectrum in a potassium bromide disc, and the same was true to a lesser extent with hydrocortisone sodium succinate.

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